A preparation method for gall midges

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The method described here concerns preparation of gall midges for light microscopic examination. The procedure is largely discussed separately for pupal skins, larvae, and adult midges. For mounting, a device is used that permits examination from both sides of the mount even at high magnification.

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Mounting of gall midge specimens in Canada balsam on ordinary microscopic slides is a method recommended e.g. by Barnes (1946) and Gagné (1989). Other media have also been used for mounting gall midges, e.g. de Faure's fluid by Edwards (1937) and Berlese's fluid by Panelius (1965). As an alternative, the preparation method referred to below was developed and found to be advantageous from several points of view.

This method, designed for the production of mounts for light microscopic examination, has long been used in connection with the establishment of a gall midge collection. Today this collection, based on material gathered by E. S. and now kept at the Swedish Museum of Natural History in Stockholm, contains roughly 9000 specimens (distributed among several hundreds of species), most of them prepared according to this method. With few exceptions, specimens have been prepared from material stored in 70 % alcohol.

The mounting procedure includes a technique used by nematologists. Each object is placed between two cover glasses, the lower one rectangular and inserted between two cardboard pieces in an aluminium holder (Figs 1, 2). Thus, this device permits examination from both sides of the mount even at high magnification.

Each object is mounted in Hoyer's medium (pulverized gum arabic 30 g, distilled water 50 g, chloral hydrate 200 g, glycerol 20 g). As for preparation of this fluid cf. Martin (1977:170). The medium is soluble in water, and therefore remounting, if desirable for some reason, can be done easily.

However, in order to make the wing veins better discernible than in Hoyer's medium, one of the two wings is mounted dry (see below).

Initially the preparations were sealed but later this was found to be unnecessary.

The preparations are durable. For example, mounts made in the early 1970's are still in a good condition.

Preparation procedure

Pupal skins

The skins are transferred to a small quantity (3–4 droplets) of 50 % chloral hydrate. After about half an hour, the skins are cleaned under the binocular by means of a needle and a small knife. It is often advisable to store the skins in the chloral hydrate medium until the next day before the final cleaning is performed. A slight warming over a spirit flame may also be helpful but has to be done with caution in order to avoid "explosion". After cleaning, the skins are mounted, usually in dorsoventral position, in Hoyer's medium.

Larvae

The procedure is largely the same as that mentioned above for pupal skins. Each specimen is, however, either cut transversely in two pieces or a slit is made in or near the border-line between abdominal segments IV and V, for example. Then, while the specimen is hold fast with a needle under the binocular, the body content is pressed and pumped out by means of the flat side of a small knife. Several transfers to 50 % chloralhydrate is

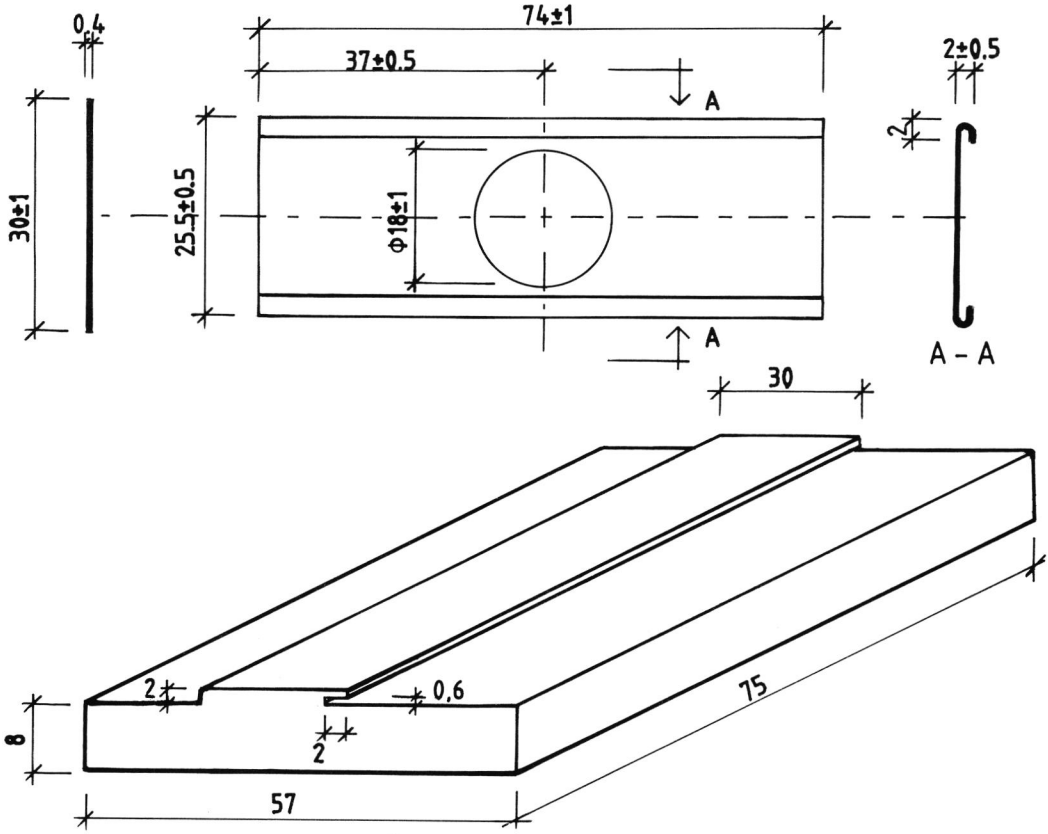

Fig. 1. *Above*: The aluminium holder. The vertical line to the left refers to the aluminium plate before production of the rims. *Below*: Iron tool for production of the rims of the holder. The aluminium plate is placed with one long side in the slit, and is then bent upwards and downwards. This operation is repeated with the opposite side in the slit. Measurements in mm.

Upptill: Aluminiumhållaren. Vertikala linjen till vänster hänför sig till aluminiumplattan före tillverkning av falsarna. *Nedtill*: Järnverktyg för framställning av hållarens falsar. Aluminiumplattan placeras med ena långsidan i skåran, böjs därefter uppåt-nedåt. På samma sätt förfars med motstående sida i skåran. Mått i mm.

advisable. Slight warming (see above under pupal skins) may also be useful. When clean the skins are mounted dorsoventrally in Hoyer's medium.

Adults

The specimen is placed in a dish with 70 % alcohol. By means of a knife and pair of forceps the right wing is removed and transferred on a brush to a droplet of 70 % alcohol on the rectangular glass in the mounting device. When dry, i.e. after evaporation of the alcohol, the wing is covered by a cover glass, and this latter finally glued to the rectangular glass.

The remainder of the specimen is dissected in 50 % chloral hydrate. The head, the left wing, the thorax including halteres and legs, and the abdomen are separated from each other. The male terminalia are disconnected from the rest of the abdomen. To get the female abdomen sufficiently clear it is advisable to store it in 50 % chloral hydrate for several hours, sometimes until the next day. Apart from this, transfer to Hoyer's mounting medium can be performed immediately. It is to be recommended that no more than one or two objects are mounted under one and the same cover glass (Fig. 2). The head is placed so that it can be examined in frontal view. Regarding the abdomen

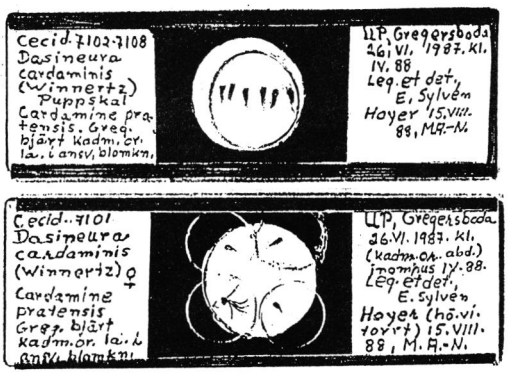

Fig. 2. Mounts. The upper one with pupal skins, the lower one with the separated parts of an adult midge. Cardboard, 1 mm thick. Upper cover glass, ø 10 mm. Lower cover glass, 24×32 mm.

Preparat, det övre med puppskal, det nedre med de separerade delarna av en fullbildad mygga. Pappskiva, 1 mm tjock. Övre täckglas, ø 10 mm. Undre täckglas, 24×32 mm.

and the male terminalia, a dorsoventral position is usually preferable.

Discussion

Chloral hydrate is classified as a poison and therefore has to be used with caution. Especially in long-termed preparation work it is advisable to perform the dissections etc. in a ventilated hood, for example. Concerning the dissection and cleaning procedures it is also feasible to use, instead of 50 % chloral hydrate, e.g. 70 % alcohol (dissection of adult specimens) or lactic acid (cleaning of pupal and larval skins). However, after treatment in lactic acid, in order to avoid crystals in the preparations, careful rinsing in distilled water has to be carried out.

It is frequently not suitable to prepare adult specimens that have been stored in 70 % alcohol for only a short time. A storage for a period of about 3–24 months is to be recommended. Several years storage may cause specimens to deteriorate more or less completely.

Objects removed from Hoyer's medium, even if they have been kept in the mounts for several years, are usually well suited for investigation in SEM. This was recently discovered and found to be extremely valuable in connection with a structural study of female abdominal features in certain gall midges (Sylvén & Tastás-Duque, in prep.).

In principle, the mounting technique described above is probably suitable also for various other kinds of small insects.

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Sammanfattning

Metoden avser preparering av spritkonserverade exemplar av gallmyggor för ljusmikroskopisk undersökning. Höger vinge monteras torr men i övrigt förbehandlas objekten i 50 % kloralhydrat samt monteras i Hoyer's kloralhydratmedium. I det färdiga preparatet är varje objekt placerat mellan två täckglas, det undre rektangulärt samt infogat mellan två pappskivor i en aluminiumhållare (Fig. 1, 2). Anordningen tillåter även vid stark förstoring undersökning från preparatets båda sidor.